D-110

DATA ASSESSMENT REPORT

Technical Directive: 05
Technical Directive Title: Reilly Tar Technical Support

Technical Directive Report

by

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OBJECTIVE AND SCOPE

Water samples in the area of St. Louis Park are contaminated with coke tar distillate consisting of a mixture of volatile organic compounds called creosote. Twenty one polycyclic compounds and 49 nitrogen containing heterocyclics have been identified from the contaminated water. There are undoubtedly hundreds of other potential toxicants in these samples. However, this document is confined to discussing the commonality of metabolic activation of polycyclic hydrocarbons to carcinogen forms which are identical in humans and laboratory animals indicating that laboratory studies dealing with the carcinogenicity of these compounds can be extrapolated to assessing potential risk to humans. Also, this report shows that mixtures of polycyclic carcinogens and non-carcinogens even at low concentration of individual components may act synergistically to present a greater tumor forming potential than the sum of the individual components of the mixture tested separately.

INTRODUCTION

Polycyclic hydrocarbons are formed by the incomplete combustion of carbonaceous material and are major components of coal tar, exhaust gases from transportation sources and from refuse burning (Committee on Biological Effects of Atmospheric Pollutants 1972). Temperatures under 1000°C allow free-radical polymerization of carbon to build various polyaromatic structures in the most energetically stabilized forms.
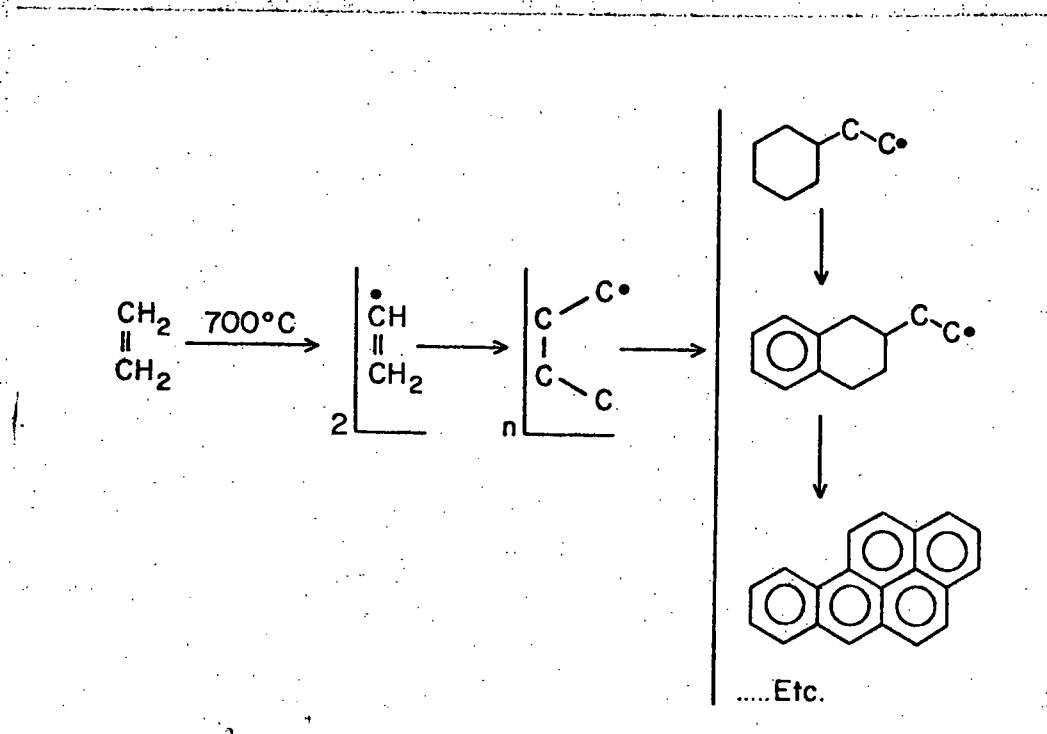

Figure 1   Free-Radical Polymerization.

Figure 1 shows the possible pyrolytic formation of benzo(a)pyrene via ethylene free-radical conjugation (Badger 1962).

Benzo(a)pyrene has been the most extensively studied carcinogenic polycyclic hydrocarbon and has become the prototype molecule to attempt an understanding of the metabolic basis for the substantial tumorigenic capability of members of this class of chemicals (Gelboin and Ts'o 1978-1982).

Polycyclic hydrocarbons are quite hydrophobic due to their lack of functional groups and the high resonance stabilization caused by the pi-electron cloud over the asymmetric annellation of the constituent benzene rings (Clar 1964). Therefore, the major functions of the detoxification process are two fold; first to deactivate the toxic functional groups on the molecule, and secondly to add on to or change the molecule in some fashion to render it water soluble for more facile excretion as a harmless waste product (e.g. addition of

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-OH; glucuronic acid; sulfate).

Polycyclic hydrocarbons are also quite inert chemically due to the aforementioned resonance stability and require fairly vigorous reaction conditions in the laboratory to place functional groups on the parent molecules. This chemical stability is the reason for their long term survival in the environment without substantial degradation and is one of the main reasons polycyclic hydrocarbons present a very substantial hazard to the community surrounding the former creosote plant in St. Louis Park, Minnesota.

BIOTRANSFORMATION

Once these chemicals enter the body, the natural detoxifying systems become operable (Selkirk 1980). However, in attempting to "defuse" the carcinogen the body is inadvertantly forming the active species of the polycyclic molecule (Selkirk and MacLeod 1982). This can best be visualized using an activation-energy diagram, wherein the steps in the metabolic activation scheme are superimposed on the energy curve to exemplify the requirement of the inert parent molecule to be raised to a higher energy level.
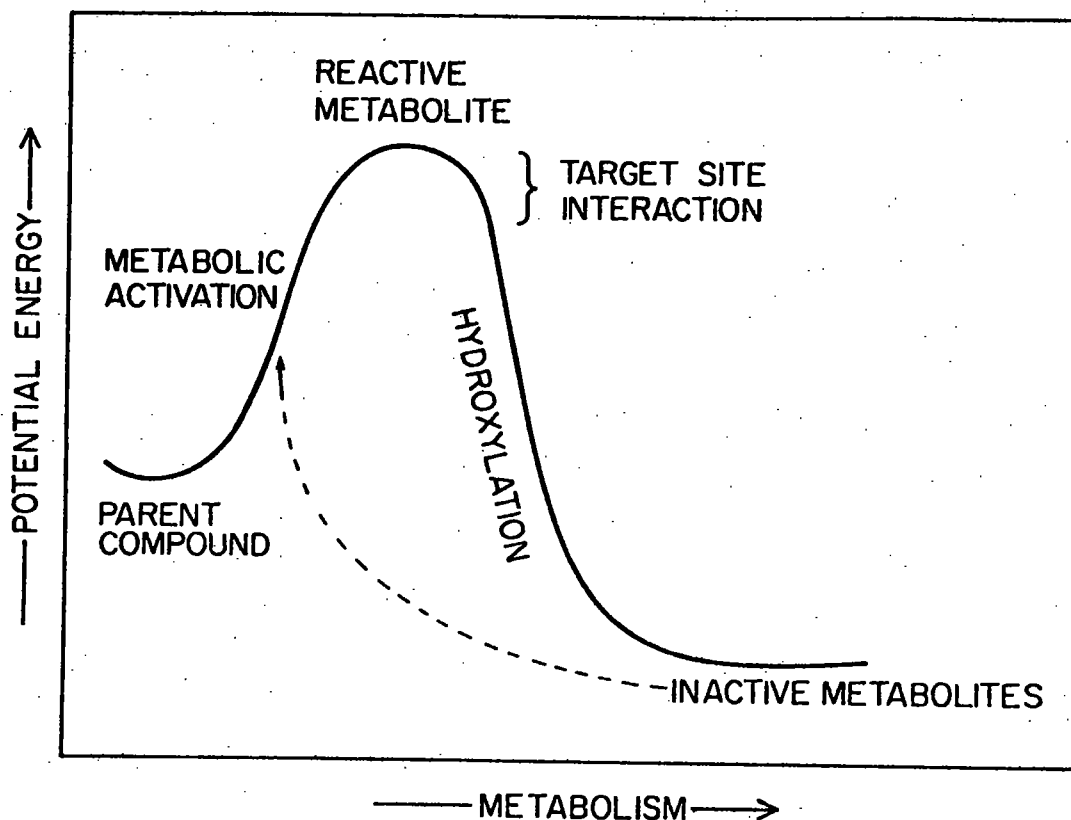
Figure 2   Metabolic Activation of Chemical Carcinogens.

Hydroxylation is one of the most common detoxifying mechanisms, and in or accomplish the addition of this chemical group, cellular drug

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detoxifying system must first activate the carcinogen molecule to allow
the breakdown steps to proceed to final excretion products. Therefore,
the inactive parent molecule is activated to a short-lived intermediate
which is transformed into a water soluble hydroxyl product for con-
jugation which is followed by excretion via the bladder or feces (in-
active metabolites). However, polycyclic hydrocarbons such as those
found in the Reilly Tar samples (e.g. benzo(a)pyrene) form sufficiently
long lived intermediates within the cell (reactive metabolite) that
reaction with critical target sites within the cell such as the genetic
material (DNA, RNA) can occur. This reaction (target site interaction)
is known to lead to cell death, mutation, cancer, and teratogenesis.
The dotted line in Figure 2 represents a relatively unique situation
for polycyclic carcinogens where some inactive metabolites (hydroxy-
lation products) that have failed to damage the cell can be recycled
for another round of activation to form an even more potent toxicant
with greater mutagenic and carcinogenic activity than the first reactive
intermediate (Slaga et al. 1976). This is best illustrated with the
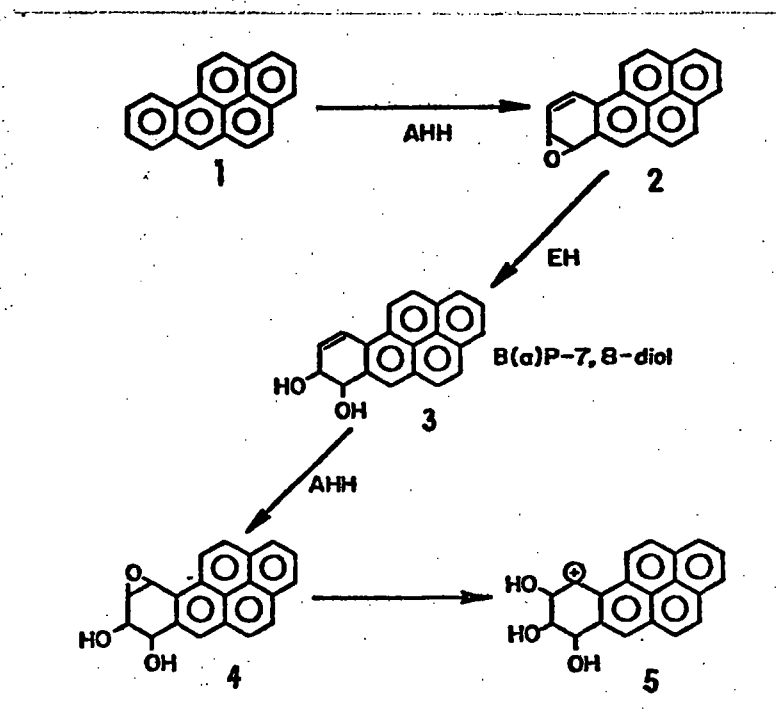activation pathway for benzo(a)pyrene.

Figure 3   Activation of Benzo(a)pyrene to its Carcinogenic Form.

Figure 3 shows benzo(a)pyrene (1) being activated by the drug
metabolizing enzyme complex AHH (aryl-hydrocarbon hydroxylase) to a
reactive epoxide intermediate (2). This epoxide is detoxified to an
inactive hydroxyl product (3). However, the inactive dihydrodiol (3)
is reactivated by the same enzyme complex to the most carcinogenic
form of benzo(a)pyrene; namely a diol-epoxide (4). This highly un-
stable epoxide metabolite will spontaneously open the ring to form a
triol-carbonium (5). This final intermediate, which possesses a
positive charge (+), reacts with critical areas in the cell to produce
malignant transformation (Mager et al. 1977). The mechanism of
transformation is not yet understood.

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There are two reasons for detailing the metabolic activation processes in this document which relate closely to evaluating the potential hazard in the St. Louis Park area. In the first instance all known chemical carcinogens are electrophilic; their putative final reactive form is a positively charged (+) species. Also, all polycyclic hydrocarbons that have been metabolically studied produce epoxide intermediates and hydroxylated and oxygeniated end products. Of the twenty-one compounds identified in the water from test wells around the St. Louis Park, at least seven are known to follow this reactive pathway: anthracene (Akhtar et al. 1979), chrysene (Vyas et al. 1981), benzo(a)pyrene (Selkirk 1980), benzo(e)pyrene (MacLeod et al. 1979), phenanthrene (Vyas et al. 1981), dibenzanthracene (MacNicoll et al. 1979) and benzanthracene (Thakker et al. 1979). The remaining compounds have not been available in sufficient quantities to enable metabolic analysis. However, in light of the fact that all polycyclics studied to date follow this pathway, it is reasonable to assume that the remaining polycyclics in Table I form similar reactive intermediates.

It is also important to realize that this activation pathway, including the mechanism of action of the monooxygenase enzyme system and the metabolites formed, is identical in all species that have been studied – including humans (Selkirk et al. 1976). Metabolite profiles seen in laboratory test animals such as mice, rats, hamsters and monkeys are qualitatively identical to human with some variance in metabolite ratios.(Selkirk 1977). The major difference between species which are relatively susceptible to malignant transformation and those relatively resistant, may be a function of metabolic rate; whereby cells resistant to malignant transformation are more efficient in removing activated intermediates than are susceptible cells

CARCINOGENICITY AND MUTAGENICITY OF POLYCYCLIC HYDROCARBONS IN ST. LOUIS PARK WATER

The twenty-one compounds identified from water samples between 1978 - 1979 have been surveyed for carcinogenic and mutagenic activity in the scientific literature. Table I summarizes this data and represents experimental evidence in rodents and in human and rodent cells. While these compounds represent a small fraction of the polycyclic molecules in creosote they include a number of known carcinogens and mutagens, and thus form an adequate cross-section to form a hazard assessment. In addition to testing of the specific compounds, coke tar gas condensate itself has been tested directly for skin tumor activity in mice (Yanysheva et al. 1964). At the end of sixteen months all surviving animals were tumorous.

Table I was compiled from the Registry of the Toxic Effects of Chemical Substances (RTECS, Chemical Information Service, Washington, D.C.) and represents an up to date survey. However, additional data searches of the most recent literature in the Toxline Data Base of the National Institutes of Health confirm this survey. (The extensive referencing of the literature confirming the results in Table I will be supplied as an appendix to this report.)

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TABLE I   Carcinogenic and Mutagenic Activity of Compounds Found in
St. Louis Park Water From Test Wells.

Compound	Carcinogen	Mutagen
Biphenyl	+	+
Anthracene	+	+
Benzanthracene	+	+
Chrysene	+	+
Benzo(a)pyrene	+	+
Phenanthrene	+	+
o-Phenylenepyrene	+	+
Pyrene	±	+
Fluoranthene	±	+
Benzo(e)pyrene	±	+
Benzo(k)fluoranthene	±	nt*
Benzo(j)fluoranthene	±	nt*
2-methyl-naphthalene	(data incomplete)	
Acenaphthalene	"	"
Fluorene	"	"
1,2,6,7-tetrahydropyrene	"	"
9,10-benzphenanthrene	"	"
Benzo(g,h,i)perylene	"	"
Naphthacene	"	"
Dibenz(a,c)anthracene	"	"

* nt = not tested

ST. LOUIS PARK WATER AS A HEALTH HAZARD

   The ground water surveys in the St. Louis Park area have shown
the land in the immediate area of the former Reilly Tar Company to be
highly contaminated with organic soluble and base soluble chemicals.
The degree of contamination in the various water samples falls between
unpleasant taste and smell to pure pools of chemicals that from a two-
phase system. The density of the organic chemicals result in vertical
percolation through the substrata into the aquafers which supply
drinking water to the surrounding community.

   The concentration of various polycyclic hydrocarbons identified in
the water samples are a function of their relative proportion in the
creosote, but likely follow an adsorption chromatography effect caused

by the packed soil and clay and the relative solubility of each
polycyclic hydrocarbon in water. Changes should be expected in the
concentrations of the different polycyclics with time, when the more
hydrophobic compounds (e.g. benzo(a)pyrene) will increase.

SYNERGISTIC EFFECT OF MULTIPLE CARCINOGENS AND MUTAGENS

It is quite apparent that anyone drinking creosote contaminated
water is exposed to a battery of known and suspected carcinogens and
mutagens. While some potent carcinogens are present in low concen-
tration their biological effects are known to be potentiated by
the presence of other polycyclic hydrocarbons and/or other chemicals
that increase the level of the monooxygenase enzyme complex, causing
a further increase in the body tissue's ability to activate carcino-
gens to their reactive forms. This effect was clearly demonstrated
in a recent study in which auto exhaust emissions, coke oven tar and
roof tar were tested for skin tumor forming activity in mice, and
compared against the relative content of benzo(a)pyrene in each test
substance (Nesnow et al. 1982).

In the case of roof tar, tumor yield was approximately six-fold
greater and coke oven main produced an eight-fold greater tumor yield
than was produced by an equivalent amount of benzo(a)pyrene in each
tar sample. Undoubtedly the potentiation effect was a function of
multiple initiators and promoters.

The biochemical mechanism of tumor induction and enhancement is
not well understood and demands an extremely conservative approach
to a "threshold concept" or a "minimal acceptable dose" allowance.
While it is experimentally possible to apply low enough concentrations
of carcinogen to form no tumors in test animals, these experiments
are always done with pure and well defined chemicals and under care-
fully controlled laboratory conditions.

Our experimental knowledge of animal treatment with mixtures of
chemicals, both carcinogens and mutagens, is quite rudimentary at the
present time. In addition, the composition of environmental pollutants
from industrial sources such as creosote plants is non-uniform and
precludes any attempt at complete analysis. These uncertainties,
coupled with the tumor enhancing capability of carcinogen mixtures,
make impossible the establishment of an acceptable level of ingested
carcinogen that could be considered relatively safe for all people.

An additional series of toxic chemicals have been identified in
one report (Pereira et al. U.S. Geological Survey April, 1982). These
are nitrogen containing heterocyclics. Nitro-arenes and aza-arenes
most certainly contribute to the carcinogenic and mutagenic hazard
in the St. Louis Park water. While the mutagenic and carcinogenic
potential of these toxic chemicals have not been studied in the same
depth as polycyclic hydrocarbons, three compounds of the forty-nine
identified in the water samples are potent carcinogens and mutagens.
The amino-arene, 2-naphthalenamine was one of the first compounds im-
plicated in bladder cancer found in workers in the German dyd stuffs
indistry (Rehn 1895). In addition, o-toluidine and quinoline are
potent rodent carcinogens and mutagens.

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REFERENCES

Committee on Biological Effects of Atmospheric Pollutants. 1972.
Particulate Polycyclic Organic Matter. Wash. D.C.: Nat'l. Acad.
of Sci. (USA), pp 13-35.

Badger GM, 1962. Nat'l. Cancer Inst. Monogr. 9. Wash. D.C.: U.S.
Gov't. Printing Office, pp 1-16.

Gelboin HV, Ts'o POP, eds. 1978-1982. Polycyclic Hydrocarbons and
Cancer. New York: Acad. Press, 3 vols.

Clar E. 1964. Polycyclic Hydrocarbons, Vol. 1. New York: Academic
Press, pp 70-85

Selkirk JK. 1980. Chemical Carcinogenesis: A Brief Overview of
the Mechanism of Action of Polycyclic Hydrocarbons, Aromatic Amines,
Nitrosamines and Aflatoxins. Modifiers of Chemical Carcinogenesis.
Slaga T, ed. New York: Raven Press, pp 1-31

Selkirk JK, MacLeod MC. 1982. Chemical Carcinogenesis: Nature's
Metabolic Mistake. Bioscience 32: 21-25

Slaga TJ, Vaije A, Berry DL, Bracken WM. 1976. Skin Tumor Initiating
Ability of Benzo(a)pyrene, 4,5-,7,8-, and 7,8-diol-9,10-
epoxide and 7,8-diol. Cancer Lett. 2: 115-112

Mager R, Huberman E, Yang SK, Gelboin HV, Sachs L. 1977.
Transformation of Normal Hamster Cells by Benzo(a)pyrene Diol-
Epoxide. Int. J. Cancer 19: 814-817

Akhtar MN, Hamilton JG, Boyd DR, Braunstein A, Seifried HE, Jerina DM.
1979. Anthracene-1,2-oxide: Synthesis and Role in the Metabolism of
Anthracene by Mammals. J. Chem. Soc. (Perkin I): 1442-1446

Vyas KP, Yagi H, Levin W, Conney AH, Jerina DM. 1981. Metabolism of
(-)-trans(3R,4R)-dihydroxy-3,4-dihydrochrysene to diol-epoxides by
Liver Microsomes. Biochem. Biophys. Res. Commun. 27: 961-969

MacLeod MC, Cohen GM, Selkirk JK. 1979. Metabolism and Macro-
molecular Binding of the Carcinogen Benzo(a)pyrene and its Relatively
Inert Isomer Benzo(e)pyrene by Hamster Embryo Cells. Cancer Res. 39:
3463-3470

MacNicoll AD, Burden PM, Rattle H, Grover PL, Sims P. 1979. The
Formation of Dihydrodiols in the Chemical or Enzymic Oxidation of
Dibenz(a,c)anthracene, Dibenz(a,h)anthracene and Chrysene.
Chem. Biol. Interact. 27: 365-379

Thakker DR, Levin W, Yagi H, et al. 1979. Metabolism of Benzo(a)-
anthracene to its Tumorigenic 3,4-dihydrodiol. Mol. Pharmacol 15:
138-153

Selkirk JK, Yang SK, Gelboin HV. 1976. Analysis of Benzo(a)pyrene
Metabolism in Human Liver and Lymphocytes and Kinetic Analysis of
Benzo(a)pyrene in Rat Liver Microsomes. Carcinogenesis. Vol. 1

Freudenthal RI, Jones PW, eds. New York: Raven Press, pp 153-169.

Selkirk JK, MacLeod MC, Moore CJ, Mansfield BK, Nikbakht PA, Dearstone KC. 1982 . Species Variance in the Metabolic Activation of Polycyclic Hydrocarbons. Mechanism of Chemical Carcinogenesis. New York: Alan R. Liss, Inc. (in press).

Yanysheva NYa, Andrienko LG, Balenko NV, Kireeva IS. 1964. The Carcinogenic Properties of Coke Oven Wastes. Hygiene and Sanitation 29: 6-11.

Nesnow S, Evans C, Stead A, Creason J, Slaga TJ, Triplett LL. 1981. Skin Carcinogenesis Studies of Emission Extracts. Toxicological Effects of Emissions From Diesel Engines. Lewtas J ed. New York: Elsevier pp 295-320.